Supplementary Material

To the paper: Screening for synthetic lethal mutants in *Escherichia coli* and identification of EnvC (YibP) as a periplasmic septal ring factor with murein hydrolase activity, by T. Bernhardt & P. de Boer.

Detailed Experimental Procedures

Plasmid and phage constructs

Plasmids pET21b (Novagen), pSC101 (Bernardi and Bernardi, 1984), pZAQ (Ward and Lutkenhaus, 1985), pMLB1113, pDB170 and pRC7 (de Boer *et al*., 1989), pDR107b (Raskin and de Boer, 1999), pAB10 (Hale *et al*., 2000), pKD13, pCP20 and pKD46 (Datsenko and Wanner, 2000), pJE80 (Johnson *et al*., 2002), and pCH151, pTB28, pTB29, pTB32, and pTB37 (Bernhardt and de Boer, 2003), were described previously.

With the exceptions of pDR140, pTB8, and pTB63 all of the plasmids constructed for this study are derivatives of the medium copy vector pMLB1113 [*bla lacI*^{q} P_{lac}::*lacZ*] (de Boer *et al*., 1989). In all cases where *gfp* was used, the allele is *gfpmut*2 (Cormack *et al*., 1996). Unless indicated otherwise, Vent DNA polymerase (NEB) was used according to the instructions to produce PCR fragments and MG1655 chromosomal DNA was used as the template for PCR. Where appropriate, the restriction enzyme sites or overhangs used for cloning are underlined in the primer sequences.

For pTB5 [P_{lac}::^{ss}*torA*], the first 43 codons of *torA* were amplified in two reactions using either 5'AATTAACTCAGGAGATATACCATGAACAATAACGATCTCTTTCAGGC3' or 5'AACTCAGGAGATATACCATGAACAATAACGATCTCTTTCAGGC3' as the forward primer and 5'CGCGGATCCCCCGGGAAGCTTCTCGAGGTCGACGAATT CCGCCGCTTGCGCCGCAGTCG3' as the reverse primer. The resulting 186bp and 182bp fragments were mixed, incubated in a boiling water bath, and cooled, generating a population of fragments with an *EcoR*I overhang on one end. These were digested with *Hind*III and ligated with pMLB1113 (de Boer *et al*., 1989) that had been digested with *EcoR*I and *Hind*III.

For pTB6 [P_{lac}::^{ss}*torA-gfp-t*], *gfp* fused to the T7-tag (Novagen) at its 3' end was amplified from pDR107b (Raskin and de Boer, 1999) using either 5'AATTTATGAGTAAAGGAGAAGAACT TTTCACTGG3' or 5'TATGAGTAAAGGAGAAGAACTTTTCACTGG3' as the forward primer and 5'GCGCGCAAGCTTCCCGGGGGATCCCTCGAGGTCGACGAATTCACCCATTTG CTGTCCACCAGTC3' as the reverse primer. The resulting 797 and 793bp fragments were annealed as described above, digested with *Hind*III, and ligated with pTB5 digested with *EcoR*I and *Hind*III.

For pTB8 [mini-F ori; *bla lacI*^{q} P_{lac}::*minCDElacZ*], *minCDE* was amplified from pDB170 (de Boer *et al*., 1989) using the primers 5'TTAGGCACCCCAGGCTTTACAC3' and 5'GCGCGCAAGCTTGGGCTTATTTCAGCTCTTCTGCTTCCG3'. The resulting 1959bp fragment was digested with *EcoR*I and *Hind*III and ligated with similarly digested pRC7 (de Boer *et al*., 1989), itself a derivative of the mini-F vector pFZY (Koop *et al*., 1987).

For pTB25 [P_{lac}::*envC*], *envC* was amplified using the primers 5'CTCTGAATTCTCTAGACCGACCATGTTGTCGCTGATGG3' and 5'CTCTAAGCTTGTCGACAGGAGAGCGCCAACAGAGCGGC3'. The resulting 1458bp fragment was digested with *EcoR*I and *Hind*III and ligated with appropriately digested pMLB1113 (de Boer *et al*., 1989).

For pTB26 [P_{lac}::*envC-gfp*], *envC* was amplified using the primers 5'CTCTGAATTCTCTAGACCGACCATGTTGTCGCTGATGG3' and 5'CCGTCTCGAGTCTTCCCAACCACGGCTGTGGAT3'. The resulting 1392bp fragment was digested with *Xba*I and *Xho*I and ligated with appropriately digested pCH151 (Bernhardt and de Boer, 2003).

For pTB43 [P_{lac}::^{ss}*amiC-envC-gfp*], *envC* was amplified with 5'GTCTCTGCTAGCTGATGAGCGTGACCAACTCAAATC3' and 5'CCGTCTCGAGTCTTCCCAACCACGGCTGTGGAT3'. The resulting 1168bp fragment, containing *envC* save its first 34 codons, was digested with *Nhe*I and *Xho*I and ligated with appropriately digested pTB37 (Bernhardt and de Boer, 2003). The linker sequence between EnvC and GFP encoded by this construct is LEDPPAEF.

For pCH215 [P_{lac}::*mreB-gfp*], *mreB* was amplified with 5'CGACTCTAGAACAGCTTTCAGGATTATCCCTTAGTATG3' and 5'CGCTTCTCGAGCTCTTCGCTGAACAGGTCGCCG3'. The resulting 1077bp fragment was digested with *Xba*I and *Xho*I and ligated with appropriately digested pCH151 (Bernhardt and de Boer, 2003). The linker sequence is the same as for pTB43.

For pFB115 [P_{lac}::*mreB-gfp*], the primers 5'TCGAGACGGTTGAAAGCACCGGTAAAGTG3' and 5'GATCCACTTTACCGGTGCTTTCAACCGTC3' were annealed and ligated with pCH215 digested with *Xho*I and *BamH*I. This changes the linker sequence between MreB and GFP from LEDPPAEF to LETVESTGKVDPPAEF. The underlined sequence is a linker derived from MinC.

For TB46 [P_{lac}::^{ss}*amiC-gfp*], ^{ss}*amiC* was amplified from pTB28 (Bernhardt and de Boer, 2003) with 5'CCTGTCTAGAAACTCAGGAGATATACCATGTCAGGATCCAACACTGCAAT3' and 5'TCTCCCTCGAGACCAGATCTCGCAGCTAGCGAGACCTGACTTACGCTCAATAGCC3'. The resulting fragment was digested with *Xba*I and *Xho*I and ligated with appropriately digested pFB115.

For TB47 [P_{lac}::^{ss}*amiC-envC-gfp*], the 1271bp *Xba*I-*Xho*I fragment from pTB43 was used to replace the 1071bp XbaI-XhoI fragment of pFB115, thus changing the linker between EnvC and GFP. We found ^{ss}AmiC-EnvC-GFP fusions with the pFB115 MinC-derived linker (see above) to be more stable than those with the pTB43 linker (data not shown).

For pDR140 [P_{T7}::*tfkh*], which encodes a fusion of the T7 (T), Flag (F), heart-muscle kinase (K), and 6xHis (H) tags (see below) downstream of the T7 promoter, a 1684bp fragment of pAB10 (Hale *et al*., 2000) encoding the FKH tag was amplified with 5'GCCCTCGAGATGGACTACAAAGACGATG3' and 5'CTGAAGCTTACCAATGCTTAATCAGTGAGGC3'. The fragment was digested with *Pst*I and *Xho*I and ligated with appropriately digested pET21b (Novagen).

For pTB57 [P_{T7}::*envC-fkh*], which encodes EnvC with its first 34 a.a. replaced with the tripeptide MAS and with the FKH-tag fused to its C-termius (Fig.3B), *envC* was amplified with 5'GTCTCTGCTAGCGATGAGCGTGACCAACTCAAATC3' and

5'CCGTCTCGAGTCTTCCCAACCACGGCTGTGGAT3'. The resulting 1167bp fragment was digested with *Nhe*I and *Xho*I and ligated with appropriately digested pDR140.

For pTB58 [P_{lac}::*ybeBA pbpA rodA*] and pTB59 [P_{lac}::*pbpA rodA*], the *ybeBA pbpA rodA* locus was amplified with 5'CTCTGAATTCGCCTCTGGTTCAGGTATACTGACAGACC3' and 5'CCTCTAAGCTTCTGCTTACGCATTGCGCACCTC3' generating a 3942bp fragment, and the *pbpA rodA* locus was amplified with 5'CTCTGAATTCCCGTGAGTGATAAGGGAGCTTTGAGTAG3' and 5'CCTCTAAGCTTCTGCTTACGCATTGCGCACCTC3' generating a 3099bp fragment. These fragments were digested with *EcoR*I and *Hind*III and ligated with appropriately digested pMLB1113 (de Boer *et al*., 1989).

For pTB63 [*ftsQAZ*], the 4569bp *Nhe*I-*Xho*I fragment of pZAQ (Ward and Lutkenhaus, 1985), containing the *ftsQAZ* locus and native promotors, was used to replace the 1305bp *Nhe*I-*Xho*I fragment of pSC101 (Bernardi and Bernardi, 1984). Thus, the *ftsQAZ* locus was moved from a medium-copy pBR322 backbone to a low-copy pSC101 backbone.

For pTB69 [P_{lac}::*ybeBA*], the *ybeBA* locus was amplified with 5'CTCTGAATTCGCCTCTGGTTCAGGTATACTGACAGACC3' and 5'CCTCTAAGCTTGTTCTGTAGTTTCATCCGCTGCG3' generating a 911bp fragment. The fragment was digested with *EcoR*I-*BamH*I and ligated with appropriately digested pMLB1113 (de Boer *et al*., 1989).

Phages λCH151, λTB6, λTB46, and λTB47 were obtained by crossing pCH151, pTB6, pTB46, and pTB47, respectively, with λNT5 (de Boer *et al*., 1989).

Bacterial strains

Bacterial strains used in this study are listed in Table 1 of the main text. Strains TB12, TB13, TB14, TB27, and TB55 were constructed using λRed-mediated recombineering (Datsenko and Wanner, 2000; Yu *et al*., 2000). The symbol <> denotes DNA replacement (Yu *et al*., 2000), and *frt* a scar sequence remaining after eviction of

the *aph* (Kan^R) cassette by FLP recombinase (Datsenko and Wanner, 2000). TB12 is the intermediate *lacIZYA<>aph* strain resulting directly from the λRed-mediated recombineering used for TB28 construction (Bernhardt and de Boer, 2003). TB13 is a TB12 derivative in which the *aph* cassette has been evicted.

For TB14, the *aph* cassette from pKD13 was amplified with the primers 5'AATAAGAACAATCAAAAGTACGACGGCAATGGGTTGATTGATTCCGGGGATCCGTCGACC3' and 5'AGGGGGAGGTGGAAATAGCAATGAGGAGTATCAGCAAGAAGTGTAGGCTGGAGCTGCTTCG3'. The resulting 1345bp fragment was flanked by 40bp of sequence homologous to the *min* locus (underlined in primer sequences). This fragment was electroporated into TB13/pKD46 and recombinants, in which the *minCDE* locus (from 173bp upstream of *minC* to 84bp downstream of *minE*) is replaced by *aph*, were selected by plating on LB-Kan as described (Datsenko and Wanner, 2000). To obtain TB15, TB14 was cured of pKD46 and the resulting *minCDE<>aph* allele was converted to a *minCDE<>frt* allele by evicting the *aph* cassette with FLP recombinase supplied from plasmid pCP20 as described (Datsenko and Wanner, 2000).

For TB27, the *aph* cassette from pKD13 was amplified with the primers 5'TGACTGGTAAGCCGCTGTTCATCGTGGAATAATCCCTCCCATTCCGGGGATCCGTCGACC3' and 5'CAAATGCAAGAACGTTACGACGAAATGGAAACAAAACTTAGTGTAGGCTGGAGCTGCTTCG3'. The resulting 1345bp fragment was flanked by 40bp of sequence homologous to the *envC* locus (underlined in primer sequences). This fragment was electroporated into TB13/pKD46 and recombinants, in which the *envC* locus (from 1bp upstream of *envC* to its last codon) is replaced by *aph*, were selected by plating on LB-Kan as described (Datsenko and Wanner, 2000). The resulting *envC<>aph* allele of TB27 was moved to TB28 and TB57 by P1-mediated transduction to generate strains TB35 and TB58, respectively. The *aph* cassette in TB35 was evicted as described above to generate TB44.

For TB55, the (*aph araC* P_{ara}) cassette from pTB29 was amplified with the primers 5'CAAGGGTATTTTTTAAGCTATGAATCAGCGCCATTTATCAGTGTAGGCTGGAGCTGCTTC3' and 5'ACTCAATTAGCTATTAATCATCGCCAGCGCGCGATGATGTATCGCCGAATTCGCTAGCCC3'. The resulting 2601bp fragment was flanked on either side by 40 bp of sequence homologous to the region upstream of *minC*

(underlined in the primer sequences). This fragment was electroporated into DY329 as described (Yu *et al*., 2000). Recombinants, in which the *minCDE* promoter region (from 50 to 133bp upstream of the *minC* start codon) is replaced by the (*aph araC* P_{ara}) cassette, were selected on LB-Kan plates. The resulting P_{minC}<>(*aph araC* P_{ara}) allele, referred to as P_{ara}::*minCDE* in the Results section, was moved to TB28 by P1-mediated transduction to generate TB56. TB57 was generated from TB56 by eviction of the *aph* cassette as described above.

TB61 and TB62 were constructed by P1-mediated transduction of the *recA*::Tn*10* allele from DX1 to TB57 and TB58, respectively. TB75 and TB81 were constructed by P1-mediated transduction of the *slm3* allele from Slm3 to TB28 and TB57, respectively.

Arbitrary PCR

The positions of transposon insertions were determined using arbitrary PCR (O'Toole and Kolter, 1998). Chromosomal DNA was used as the template for arbitrary PCR and was prepared from the mutants and a transposon-free control strain, using the MasterPure kit from Epicentre. For the first round of amplifications, 100μL reactions were prepared containing 1μL template, 2.5μL of each primer (20μM), Arb1 (5'GGCCACGCGTCGACTAGTACNNNNNNNNNNGATGC3') and EZTnExt (5'TTGGTTGTAACACTGGCAGAGC3'), 2μl of dNTP mix (10mM each), 5μL 10x thermopol buffer (NEB), 1μL Taq DNA polymerase (NEB), and the remaining volume made up with H_2O. The reactions were first cycled 8 times between 95ºC for 30sec, 30ºC for 30sec, and 72ºC for 1.5min, and then 30 times between 95ºC for 30sec, 44ºC for 30sec, and 72ºC for 2min. A second round of amplifications was then performed using reactions prepared as above, except that 5μL of the first reaction was used as the template and the primers were substituted with Arb2 (5'GGCCACGCGTCGACTAGTAC3') and EZTnInt (5'AAGCTCTCATCAACCGTGGCGG3'). The reactions were cycled between 95ºC for 30sec, 50ºC for 30sec, and 72ºC for 2min for a total of 30 cycles. The reactions typically yielded a single small fragment or a collection of small fragments (typically 100-500bp), which were purified using Qiagen spin columns and sequenced at the Cleveland Genomics facility using the EZTnInt primer. A BLAST search (Altschul *et al*.,

1990), using the sequence located immediately outside the transposon, was used to determine the location of the transposon in the chromosome.

Purification of EnvC

Plasmid pTB57 [P_{T7}::*envC-fkh*] encodes a non-exported derivative of EnvC in which its native signal sequence (residues 1-34) is replaced by the tripeptide MAS, and its C-terminus is fused to the FKH-tag peptide MDYKDDDDKARRASVEFHIE$(H)_6$ (Hale *et al*., 2000). BL21(λDE3)/pTB57 cells were grown overnight at 30ºC in LB-Amp-0.2% glucose, diluted 1:200 into 500mL of LB-Amp-0.04% glucose, and grown at 37ºC to an OD_{600} of 0.4. IPTG was then added to 0.84 mM and the cells were grown for an additional two hours. The cells were harvested by centrifugation, washed once with 10mL cell lysis buffer (CL buffer; 50mM sodium phosphate, 300mM NaCl, 10mM imidazole; pH 8.0), and resuspended in 10mL CL buffer. The cells were broken by two passages through a French pressure cell at 10,000 lb/in^2 followed by brief sonication. The homogenate was cleared by centrifugation at 200,000 x g for one hour, frozen quickly in a dry ice-acetone bath and stored at -80ºC overnight. The homogenate was then thawed and applied to a 0.5mL column of NiNTA-agarose (Qiagen) pre-equilibrated with CL buffer. The column was washed with 5mL CL buffer containing 20mM imidazole and 0.5mL CL buffer containing 50mM imidazole. EnvC-FKH was eluted in three 0.25mL fractions of CL buffer containing 100mM imidazole. These peak fractions were pooled, dialyzed against EnvC storage buffer (50mM HEPES-KOH, 100mM NaCl, 10% glycerol; pH 7.2), and small aliquots were stored at -80ºC. Protein concentrations were determined using the non-interfering protein assay (Genotech) according to the manufacturer's instructions with bovine serum albumin (BSA) as a standard.

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